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Published in:
Kidney International

DOI:
10.1016/j.kint.2020.04.051

Publication date:
2020

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OPEN

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Canagliflozin reduced kidney disease progression in participants with type 2 diabetes in the CANagliflozin cardioVascular Assessment Study (CANVAS) Program. This analysis explored potential mediators of the effects of canagliflozin on kidney outcomes. The percent mediating effect of 18 biomarkers indicative of disease was determined by comparing the hazard ratios for the effect of randomized treatment from an unadjusted model and from a model adjusting for the average post-randomization level of each biomarker. Multivariable analyses assessed the joint effects of biomarkers that mediated most strongly in univariable analyses. The kidney outcome was defined as a composite of 40% estimated glomerular filtration rate decline, end-stage kidney disease, or death due to kidney disease. Nine biomarkers (systolic blood pressure [8.9% of effect explained], urinary albumin:creatinine ratio [UACR; 23.9%], gamma glutamyltransferase [4.1%], hematocrit [51.1%], hemoglobin [41.3%], serum albumin [19.5%], erythrocytes [56.7%], serum urate [35.4%], and urine pH [7.5%]) individually mediated the effect of canagliflozin on the kidney outcome. In a parsimonious multivariable model, erythrocyte concentration, serum urate, and systolic blood pressure maximized cumulative mediation (115%). Mediating effects of UACR, but not other mediators, were highly dependent upon the baseline level of UACR: UACR mediated 42% and 7% of the effect in those with baseline UACR 30 mg/g or more and under 30 mg/g, respectively. The identified mediators support existing hypothesized mechanisms for the prevention of kidney outcomes with sodium glucose cotransporter 2 inhibitors. Thus, the disparity in mediating effects across baseline UACR subgroups suggests that the mechanism for kidney protection with canagliflozin may vary across patient subgroups.

Kidney International (2020) **98,** 769–777; https://doi.org/10.1016/j.kint.2020.04.051

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Received 23 January 2020; revised 6 April 2020; accepted 9 April 2020; published online 27 May 2020

KEYWORDS: chronic kidney disease; diabetes; diabetic nephropathy

The CANagliflozin cardioVascular Assessment Study (CANVAS) Program reported that the sodium glucose cotransporter 2 (SGLT2) inhibitor canagliflozin reduces the risk of clinically important kidney outcomes in patients with type 2 diabetes with established cardiovascular disease or those at high cardiovascular risk.[1] SGLT2 inhibitors prevent glucose and sodium from reabsorbing in the proximal tubule,[2] which promotes glycosuria and natriuresis and results in improved glycemia, weight loss, blood pressure, and albuminuria.[3] These effects may explain individually or jointly why canagliflozin confers long-term kidney benefits, as observed in the CANVAS Program and subsequently confirmed in the Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial.[4–6]

The mechanisms of action for kidney protection with canagliflozin is an area of great interest. In epidemiologic research, mediation analyses are used to investigate the mechanism that underlies an observed effect of an intervention on an outcome.[7] The standard methodology quantifies the degree of attenuation of the treatment effect on the outcome of interest after inclusion of the mediator (i.e., biomarker) in the efficacy estimation model. Selection of biomarkers for investigation as potential mediators may be based on prior hypotheses, by systematically testing all available biomarkers, or by some combination of the two. To qualify as a potential mediator, a biomarker must be affected both by the drug under investigation and associated with the outcome of interest. However, even when these criteria are met, the change in the biomarker does not always infer a causal association.[8]

A recent mediation analysis of the effects of canagliflozin suggested that uric acid, albuminuria, and markers of plasma volume and hematopoiesis were the most important mediators for effects on heart failure.[9] Whether the same or other mediators underlie the long-term kidney protective effects of

canagliflozin is unknown. The objective of the present analyses therefore was to explore potential mediators of the beneficial effects of canagliflozin on major kidney outcomes in the CANVAS Program.

RESULTS

The potential mediating effects of 21 biomarkers were assessed, 18 of which were available for the entire CANVAS Program and 3 of which were available for participants in the CANVAS trial but not in CANVAS-Renal. For assessments of average biomarker levels, the mean number of measurements made is shown in Supplementary Table S1. The overall average number of biomarker measurements was 14, and was least for hematocrit, hemoglobin, erythrocytes, urine pH, and ketones (mean, 8 measurements during the follow-up period). The most biomarker measures were acquired for blood pressure, pulse rate, weight, and body mass index (mean, 19 measurements during the follow-up period).

Effects of canagliflozin compared with placebo on potential mediators

There were clear effects of canagliflozin, compared with placebo, on multiple potential mediators of effect (Table 1[9] and Supplementary Tables S2, S3, and S4): hemoglobin A1c, fasting plasma glucose, systolic blood pressure (SBP), diastolic blood pressure, weight, body mass index, urine pH, serum bicarbonate, serum urate, serum gamma glutamyltransferase (GGT), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, hematocrit, hemoglobin, erythrocyte concentration, serum albumin, ketonuria, and urinary albumin:creatinine ratio (UACR).

Associations of postrandomization levels of potential mediators with risk of the composite kidney outcome

For 10 of the 18 potential mediators (SBP, triglycerides, UACR, GGT, hematocrit, hemoglobin, serum albumin, erythrocytes, serum bicarbonate, and serum urate) assessed in the overall CANVAS Program, there was a significant association of the average postrandomization level with the risk of kidney outcomes in the Cox proportional hazard regression models (all $P < 0.05$) (Table 2). The supplementary analyses of the biomarkers measured only in CANVAS identified additional significant associations of the average follow-up levels of urine pH with the risk of kidney outcomes (Supplementary Table S2).

Estimated mediation of the effects of canagliflozin on kidney outcome

For all 10 (1 in CANVAS alone) biomarkers modified by canagliflozin versus placebo, there also were associations of the biomarker changes with the subsequent risk of the kidney outcome (SBP, triglycerides, UACR, GGT, hematocrit, hemoglobin, serum albumin, erythrocytes, serum bicarbonate, serum urate, and urine pH). Nine of these biomarkers were identified as individually significant mediators of the effect of canagliflozin on kidney outcome when the average postrandomization levels were assessed in the primary models (SBP, UACR, GGT, hematocrit, hemoglobin, serum albumin, erythrocytes, serum urate, and urine pH). The 3 with the largest magnitude of mediating effect were erythrocyte concentration (57%), hematocrit (51%), and hemoglobin (41%) (Figure 1). Assessment of joint effects in a multivariable model of the strongest 3 mediators representing different modes of action resulted in the inclusion of erythrocyte concentration, serum urate, and SBP, and an estimated cumulative mediation of 115% (95% confidence interval [CI], 95–159) of the effects of canagliflozin on kidney outcome (Figure 2).

For the early change in levels of potential mediators, there were significant associations between potential mediators and kidney outcome for 9 of the 18 biomarkers (Table 3 and Supplementary Table S3). The same biomarkers identified as possible mediators in the analyses of the average postrandomization levels, with the exception of serum albumin, were potential mediators in analyses of early change in the biomarker of interest, although the magnitude of the mediating effect in the early change analyses was somewhat smaller (Table 3).

Subsidiary analyses based on the alternative counterfactual framework, which aims to establish causal inferences in epidemiologic research, identified 11 mediators based on average levels (Supplementary Table S4). In the multivariable model, inclusion of the average levels of erythrocyte concentration, serum urate, and UACR resulted in an estimated overall mediation of 96% (95% CI, 92–104).

Mediating effects in patient subgroups

In subgroup analyses, mediating effects of biomarkers generally were similar across patient subgroups, except for UACR. In patients with a baseline UACR less than 30 mg/g, the mediating effect of UACR was 7.4% (95% CI, 2.8–20.1), whereas it was 42.3% (95% CI, 21.1–113.6) in patients with a baseline UACR of 30 mg/g or greater (Figure 3). When mediators were assessed jointly, erythrocytes, serum urate, and UACR were the strongest mediators in patients with a UACR less than 30 mg/g, whereas in patients with a UACR of 30 mg/g erythrocytes or greater, UACR and body mass index were the strongest mediators.

DISCUSSION

We identified a large set of potential mediators of the effect of canagliflozin on kidney outcomes. Some of the identified mediators, such as UACR, were expected given the well-established relationship between UACR changes and kidney outcomes. Weak mediating effects were observed for SBP and GGT, while hematocrit and hemoglobin, which may be markers of volume and/or hematopoiesis, were identified consistently as mediators across all analyses. Measures of glycemic control were not identified as mediators. Mediating effects of all biomarkers were consistent in patient subgroups, except for UACR. UACR showed stronger mediating effects in patients with increased UACR, suggesting that the underlying

Table 1 | Effects of canagliflozin on biomarkers that might mediate the effect of canagliflozin on kidney outcomes

Biomarker	Mean (SE) at baseline		Average difference (SE) at follow-up evaluation[a]
	Placebo	Canagliflozin	
Glycemia			
HbA1c, %	8.25 (0.01)	8.25 (0.01)	**–0.52 (0.02)**
Vascular tone			
SBP, mm Hg	136.90 (0.24)	136.44 (0.21)	**–3.91 (0.19)**
DBP, mm Hg	77.81 (0.15)	77.62 (0.13)	**–1.33 (0.11)**
Pulse rate, bpm	72.49 (0.16)	72.64 (0.14)	–0.22 (0.12)
Lipids, mmol/l			
LDL-C[b]	2.30 (0.01)	2.29 (0.01)	**0.11 (0.01)**
HDL-C[b]	1.18 (0.005)	1.18 (0.004)	**0.05 (0.003)**
Triglycerides[b]	2.03 (0.02)	2.02 (0.02)	0.02 (0.02)
Total cholesterol[b]	4.37 (0.02)	4.35 (0.02)	**0.16 (0.01)**
Kidney			
UACR, mg/g[c]	20.49 (315.41)	19.87 (288.54)	**–19% (–21%, –17%)**
Adiposity			
Weight, kg	90.01 (0.31)	90.28 (0.27)	**–2.20 (0.07)**
BMI, kg/m^2	31.97 (0.09)	31.95 (0.08)	**–0.79 (0.02)**
GGT, U/l	37.74 (0.64)	38.37 (0.58)	**–4.34 (0.57)**
Volume status and hematopoiesis			
Hematocrit, %	41.96 (0.06)	42.00 (0.05)	**2.53 (0.05)**
Hemoglobin, g/l	139.09 (0.22)	139.54 (0.19)	**7.65 (0.18)**
Serum albumin, g/l	41.40 (0.05)	41.32 (0.04)	**0.56 (0.04)**
Erythrocytes, $\times 10^{12}$/l	4.68 (0.01)	4.71 (0.01)	**0.27 (0.01)**
Indicators of acidosis/alkalosis			
Serum bicarbonate, mmol/l	23.37 (0.04)	23.33 (0.03)	**–0.34 (0.03)**
Other			
Serum urate, µmol/l	349.78 (1.47)	348.21 (1.24)	**–23.49 (1.02)**

BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; GGT, gamma glutamyltransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.

[a]Mixed-model with repeated-measures analysis using all data available before completion in patients who had baseline and follow-up measurements for the respective outcome. The model adjusted for region, baseline HbA1c, estimated glomerular filtration rate, BMI, baseline of the outcome, treatment, visit, and study subgroup (CANagliflozin cardioVascular Assessment Study [CANVAS]/CANagliflozin cardioVascular Assessment Study–Renal [CANVAS-R]).

[b]Fasting test results.

[c]Baseline data are the geometric mean (geometric coefficient of variation); differences are the percentage change from the adjusted geometric mean ratio (95% confidence interval) obtained from a mixed-model with repeated-measures analysis applied on log-transformed data.

Bold indicates a significant effect at $P < 0.05$.

mechanism of kidney protection from canagliflozin may vary by baseline UACR level.

Markers of volume status and erythropoiesis were strong mediators in all models. SGLT2 inhibition causes a modest natriuretic/diuretic effect,[10] which results in modest reductions in plasma volume and extracellular volume, as well as increases in hematocrit and hemoglobin. Markers of volume status, including hematocrit and hemoglobin, have been reported previously to be strong mediators of the beneficial effects of SGLT2 inhibitors on cardiovascular death and heart failure hospitalization.[9,11] Fluid overload has been associated with a higher risk of end-stage kidney disease in observational studies.[12,13] Correction of extracellular volume expansion through natriuresis and diuresis, resulting in amelioration of microcirculation in the kidney secondary to a reduction in venous pressure and interstitial pressure, has some credence as a mechanism by which SGLT2 inhibition may improve kidney outcomes.[14]

In addition to volume reduction, increases in hematocrit and hemoglobin also may reflect increased erythropoiesis. Dapagliflozin has been shown to transiently increase erythropoietin and red cell mass, indicating direct effects on erythropoiesis that may improve kidney tissue oxygenation.[15] Hypoxia in kidney tissues is a powerful predictor of adverse long-term kidney outcomes, and stimulation of erythropoiesis is another plausible potential pathway for kidney protection with SGLT2 inhibition.

It also is possible that reducing albuminuria with SGLT2 inhibition is another route through which long-term kidney protection is achieved. The underlying mechanism of the albuminuria-lowering effects of SGLT2 inhibitors are not completely understood, but reduction in intraglomerular pressure secondary to restoration of tubuloglomerular feedback has been proposed to play an important role based on findings in patients with type 1 diabetes.[16] Whether such findings equally apply to patients with type 2 diabetes is uncertain because more recent data have suggested that acute reductions in the glomerular filtration rate result from efferent arterial vasodilation, possibly through inhibition of prostaglandins.[17] Improvements in endothelial function or

Table 2 | Observational associations of potential mediators and the risk of the composite kidney outcomes

Effect of a 1-unit increase	HR	95% CI	*P* value
Glycemia			
HbA1c, %	1.04	0.94–1.15	0.449
Vascular tone			
SBP, mm Hg	1.02	1.01–1.03	**<0.001**
DBP, mm Hg	1.00	0.98–1.01	0.496
Pulse rate, bpm	1.00	0.99–1.01	0.884
Lipids, mmol/l			
LDL-C	1.04	0.92–1.19	0.510
HDL-C	0.69	0.46–1.04	0.074
Triglycerides	1.33	1.05–1.69	**0.017**
Total cholesterol	1.06	0.96–1.17	0.269
Kidney			
UACR, mg/g	1.81	1.69–1.93	**<0.001**
Adiposity			
Weight, kg	1.01	1.00–1.01	0.131
BMI, kg/m^2	1.02	1.00–1.04	0.073
GGT, U/l	1.34	1.13–1.58	**<0.001**
Volume status and hematopoiesis			
Hematocrit, %	0.85	0.83–0.88	**<0.001**
Hemoglobin, g/l	0.96	0.95–0.97	**<0.001**
Serum albumin, g/l	0.81	0.79–0.83	**<0.001**
Erythrocytes, $\times 10^{12}$/l	0.22	0.17–0.28	**<0.001**
Indicators of acidosis/alkalosis			
Serum bicarbonate, mmol/l	0.92	0.88–0.96	**<0.001**
Others			
Serum urate, μmol/l	1.01	1.01–1.01	**<0.001**

BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; GGT, gamma glutamyltransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.
Bold indicates a significant effect at $P < 0.05$.

endothelial glycocalyx function, which contribute to the charge-selective properties of the glomerular basement membrane, also may explain the albuminuria-lowering effects of SGLT2 inhibitors.[18] Regardless of the underlying mechanism, reductions in albuminuria have been associated consistently with reductions in long-term risk of kidney failure,[19] and decreasing albuminuria with canagliflozin also is likely to contribute to protection against decreases in kidney function.

The disparity in mediating effects across subgroups defined by baseline albuminuria suggests that the mechanism of kidney protection may vary in patients with low versus high degrees of albuminuria. The larger percentage of mediation in the subgroup of patients with higher degrees of albuminuria is consistent with the hypothesis that the mechanism of the beneficial effect of canagliflozin in these patients is through the decrease of albuminuria.[20] However, consistent kidney benefits for clinical outcomes with canagliflozin also have been observed in normoalbuminuric participants, suggesting alternate mechanisms.[21] However, we note that in all analyses the number of kidney outcomes in the low-albuminuria subgroup was low, which may have precluded proper assessment of mediating effects in this subgroup, and this finding warrants cautious interpretation.

Uric acid has been implicated in the progression of diabetic kidney disease through activation of the renin-angiotensin-aldosterone system and proinflammatory effects,[22] and observational studies have shown that uric acid may be an independent risk marker of chronic kidney disease progression.[23] Although these analyses support the notion that the long-term protective effects of SGLT2 inhibition may be mediated by reductions in uric acid, recent randomized controlled trials of uric acid decreases with allopurinol have not shown slowing of the progression of kidney function decline in patients with chronic kidney disease or in patients with type 1 diabetes.[24,25]

There was some evidence for mediation by measures of acidosis, although not specifically for ketosis. It has been hypothesized that, under persistent mild ketosis caused by SGLT2 inhibition, there may be preferential uptake of β-hydroxybutyrate by the kidney, thereby improving oxygen consumption at the mitochondrial level.[26] This may explain recent findings of improved mitochondrial function in patients with type 2 diabetes receiving dapagliflozin.[27] Imprecise measurement of ketonuria and the collection of data in the CANVAS trial alone reduced the power to test for a mediating effect of ketosis in these analyses. The observed reduction in urine pH and an associated mediating effect may be owing to effects of canagliflozin on the sodium hydrogen exchanger-3.[28,29] Activity of the sodium hydrogen exchanger-3 appears to be linked to SGLT2 activity in the proximal tubule, which may explain in part the natriuretic and diuretic effects of SGLT2 inhibition.

Overall, mediators of the effect of canagliflozin on kidney outcomes overlapped with those observed previously for heart failure.[9] This is perhaps no surprise in light of the strong interaction between kidney and heart failure. Because the beneficial effects of canagliflozin on kidney function did not mediate the effects on heart failure in our prior study, it is likely that the identified mediators for kidney and heart failure effects reflect common mechanistic pathways by which canagliflozin exerts protection against these end points. We note that the current analyses do not exclude other potential mechanisms of benefit of canagliflozin, such as reductions in oxidative stress, inflammation, and improvements in ischemic reperfusion injury or mitochondrial function, which to some extent could be even heart- or kidney-specific.[30–32]

These analyses of the CANVAS Program benefited from the large size of the data set, the high quality of trial conduct, the range of biomarkers available for analysis, the robust adjudication of kidney outcomes, and the multiple methods applied to assess mediation. However, there also were important limitations. All of these investigations were performed post hoc, and the results are best viewed as hypothesis-generating, given the multiple statistical tests performed. We were able to assess only the potential mediating effects of biomarkers that were measured during the trial, and it was not possible to assess the potential role of pathways acting through other putative mechanisms directly, such as inflammation, oxidative stress, arterial stiffness, or vascular resistance. There also were significant challenges inherent to the statistics underlying the methodologies applied. First, an inherent limitation to all mediation analyses is that it is not possible to be certain that the identified

	Events/1000 patient-years	Average level HR (95% CI)	% Mediation (95% CI)
Unadjusted		**0.60 (0.47, 0.77)**	
Adjusted for glycemia			
HbA1c	245/9957	0.60 (0.47, 0.78)	0.5 (–5.9, 11.2)
Adjusted for vascular tone			
SBP	249/10,005	0.64 (0.49, 0.82)	**8.9 (3.1, 24.0)**
DBP	249/10,005	0.60 (0.46, 0.77)	–1.5 (–6.7, 2.0)
Pulse rate	249/10,003	0.60 (0.47, 0.77)	–0.1 (–1.8, 1.1)
Adjusted for lipids			
LDL-C	231/9694	0.57 (0.44, 0.74)	–0.8 (–3.4, 1.2)
HDL-C	231/9697	0.58 (0.44, 0.75)	1.8 (–0.4, 6.3)
Triglycerides	231/9697	0.57 (0.44, 0.74)	–0.3 (–2.9, 0.7)
Total cholesterol	231/9698	0.56 (0.43, 0.73)	–1.4 (–5.1, 0.9)
Adjusted for renal effects			
UACR	237/9660	0.68 (0.53, 0.89)	**23.9 (11.4, 58.4)**
Adjusted for adiposity			
Weight	248/9999	0.61 (0.47, 0.79)	1.4 (–0.4, 5.4)
BMI	248/9986	0.61 (0.48, 0.79)	2.2 (–0.5, 9.3)
GGT	249/9992	0.62 (0.48, 0.79)	**4.1 (1.1, 12.6)**
Adjusted for volume status and hematopoiesis			
Hematocrit	224/9463	0.79 (0.60, 1.03)	**51.1 (28.1, 114.5)**
Hemoglobin	225/9516	0.74 (0.57, 0.97)	**41.3 (22.8, 86.9)**
Serum albumin	249/9992	0.68 (0.53, 0.87)	**19.5 (9.8, 49.5)**
Erythrocytes	225/9516	0.81 (0.62, 1.06)	**56.7 (31.1, 131.3)**
Adjusted for acidosis/alkalosis			
Serum bicarbonate	249/9989	0.59 (0.46, 0.76)	–3.5 (–10.5, –1.0)
Adjusted for others			
Serum urate	249/9992	0.74 (0.58, 0.96)	**35.4 (17.8, 81.3)**

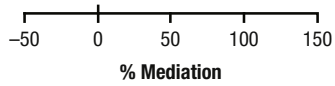

Figure 1 | Effects of univariable adjustment for potential mediators of the effect of canagliflozin on the composite kidney outcome. Bold indicates a significant effect at $P < 0.05$. BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; GGT, gamma glutamyltransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.

	Events/1000 patient-years	Average level HR (95% CI)	% Mediation (95% CI)
All			
Unadjusted		**0.60 (0.47, 0.77)**	
Adjusted for			
Erythrocytes	225/9516	0.81 (0.62, 1.06)	56.7 (36.1, 75.9)
+ Serum urate	225/9514	1.01 (0.77, 1.33)	103.0 (62.6, 204.6)
+ SBP	225/9508	1.06 (0.81, 1.40)	114.6 (94.6, 159.1)
Baseline UACR <30 mg/g			
Unadjusted		**0.50 (0.33, 0.77)**	
Adjusted for			
Erythrocytes	80/6597	0.70 (0.44, 1.10)	42.9 (22.8, 132.8)
+ Serum urate	80/6595	0.97 (0.61, 1.54)	94.3 (48.9, 227.8)
+ Log UACR	79/9509	1.06 (0.66, 1.69)	110.7 (53.1, 226.0)
Baseline UACR ≥30 mg/g			
Unadjusted		**0.64 (0.47, 0.88)**	
Adjusted for			
Erythrocytes	143/2823	0.86 (0.61, 1.21)	66.3 (34.8, 183.0)
+ Log UACR	142/2795	1.00 (0.70, 1.41)	99.1 (54.8, 225.6)
+ BMI	141/2788	1.05 (0.74, 1.49)	112.2 (60.8, 255.7)

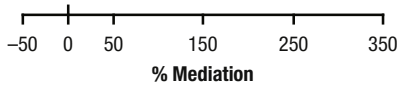

Figure 2 | Effects of multivariable adjustment for potential mediators of the effect of canagliflozin on the composite kidney outcome. Bold indicates a significant effect at $P < 0.05$. BMI, body mass index; CI, confidence interval; HR, hazard ratio; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.

Table 3 | Mediation analysis for potential mediators of the effect of canagliflozin on the composite kidney outcome when using changes measured early after randomization

Biomarker	Canagliflozin on mediators: acute change[a,b]	Association with kidney events			Mediation effect after adjustment in model			
		HR	95% CI	*P* value	Events/ patients	HR (95% CI)	Mediation, %	95% CI
Glycemia								
HbA1c, %	**−0.62 (0.02)**	1.23	1.06–1.42	**0.005**	244/9868	0.64 (0.48–0.84)	9.7	−6.4 to 40.4
Vascular tone								
SBP, mm Hg	**−3.58 (0.26)**	1.02	1.01–1.03	**0.003**	249/10,002	0.64 (0.50–0.82)	**9.5**	**2.2–24.9**
DBP, mm Hg	**−1.52 (0.15)**	1.02	1.00–1.03	0.080	249/10,002	0.61 (0.48–0.79)	3.1	−2.3 to 11.0
Pulse rate, bpm	0.06 (0.16)	1.00	0.99–1.02	0.829	249/10,001	0.60 (0.47–0.77)	−0.2	−1.8 to 0.8
Lipids, mmol/l								
LDL-C[c]	**0.09 (0.01)**	1.02	0.84–1.24	0.825	228/9460	0.55 (0.42–0.72)	−0.4	−3.0 to 2.0
HDL-C[c]	**0.04 (0.004)**	0.55	0.27–1.14	0.109	228/9465	0.56 (0.43–0.73)	2.1	−2.5 to 7.7
Triglycerides[c]	**−0.06 (0.02)**	1.45	0.99–2.12	0.060	228/9463	0.56 (0.43–0.73)	1.5	−0.6 to 4.7
Total cholesterol[c]	**0.11 (0.02)**	1.04	0.88–1.21	0.671	228/9464	0.55 (0.42–0.72)	−0.4	−3.2 to 2.4
Kidney								
UACR, mg/g[d]	**−17% (−19%, −15%)**	1.58	1.35–1.85	**<0.001**	236/9555	0.65 (0.50–0.85)	**18.2**	**3.2–47.6**
Adiposity								
Weight, kg	**−1.33 (0.04)**	1.01	0.95–1.07	0.718	248/9994	0.59 (0.45–0.76)	−4.5	−21.1 to 17.0
BMI, kg/m^2	**−0.47 (0.02)**	1.05	0.89–1.25	0.558	248/9981	0.59 (0.45–0.77)	−3.3	−20.9 to 19.2
GGT, U/l	**−3.89 (0.50)**	2.03	1.39–2.95	**<0.001**	249/9984	0.64 (0.49–0.82)	**9.3**	**1.4–23.5**
Volume status and hematopoiesis								
Hematocrit, %	**2.34 (0.07)**	0.87	0.84–0.90	**<0.001**	222/9054	0.73 (0.56–0.97)	**40.7**	**19.0–92.4**
Hemoglobin, g/l	**6.63 (0.22)**	0.96	0.95–0.97	**<0.001**	223/9146	0.68 (0.52–0.89)	**29.1**	**13.0–62.6**
Serum albumin, g/l	**0.59 (0.04)**	0.88	0.83–0.93	**<0.001**	249/9984	0.63 (0.49–0.82)	8.3	−3.3 to 24.9
Erythrocytes, $\times 10^{12}$/l	**0.26 (0.01)**	0.28	0.20–0.39	**<0.001**	223/9146	0.73 (0.55–0.97)	**40.5**	**17.9–93.3**
Indicators of acidosis/alkalosis								
Serum bicarbonate, mmol/l	**−0.42 (0.05)**	1.01	0.96–1.06	0.789	249/9981	0.60 (0.47–0.77)	−0.1	−5.0 to 4.7
Other								
Serum urate, μmol/l	**−23.21 (1.16)**	1.01	1.00–1.01	**<0.001**	249/9984	0.68 (0.52–0.87)	**19.0**	**9.5–46.7**

BMI, body mass index; bpm, beats per minute; CI, confidence interval; DBP, diastolic blood pressure; GGT, gamma glutamyltransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.
[a]Expressed as mean (SE).
[b]Mixed-model with repeated-measures analysis using all data available before completion in patients who had baseline and follow-up measurements for the respective outcome. The model adjusted for region, baseline HbA1c, estimated glomerular filtration rate, BMI, baseline of the outcome, treatment, visit, and study subgroup (CANagliflozin cardioVascular Assessment Study [CANVAS]/CANagliflozin cardioVascular Assessment Study–Renal [CANVAS-R]).
[c]Fasting test results.
[d]Baseline data are the geometric mean (geometric coefficient of variation); differences are the adjusted geometric mean ratio (95% CI) obtained from mixed-model with repeated-measures analysis applied on log-transformed data.
Bold indicates a significant effect at $P < 0.05$.

mediators are truly on the causal pathway to progression of kidney disease rather than an epiphenomenon associated with both the effects of canagliflozin and the future risk of kidney disease. Second, it is possible that the identified mediators may be reflections of progression of kidney disease, although this assumption is less likely because the mediators were affected differentially in the placebo and canagliflozin groups and mediators in analyses of early change were similar to mediators identified in analyses using the average post-randomization level. Third, our analyses were limited by their capacity to control for interactions between mediators and to provide robust estimates of uncertainty. Specific findings also were somewhat dependent on whether the standard or counterfactual approach was taken, although results were broadly similar. Assessing the joint effects of mediators resulted in more than 100% of the effect being explained by only 3 mediators, and this highlights the limited capacity to explore and control for double counting of a mechanistic pathway that is captured by more than 1 biomarker. It also is possible that there are other mechanistic pathways that were captured by none of the biomarkers included and for which the magnitude of the mediating effect remains entirely unknown. It should be noted that the composite kidney outcome was driven by the sustained 40% decrease in the estimated glomerular filtration rate component, and there were few occurrences of end-stage kidney disease.

In conclusion, we identified a diverse set of potential mediators of the effect of canagliflozin on kidney outcomes. Some mediating effects were anticipated, others were not. Our analyses provide some support for most of the hypothesized mechanisms for the prevention of adverse kidney outcomes with SGLT2 inhibitors.

METHODS

The CANVAS Program integrated data from 2 randomized trials (CANVAS and CANVAS-Renal) comparing the effects of canagliflozin versus placebo. The trials were scheduled for joint closeout and analysis when at least 688 cardiovascular events had been observed. All participants provided written informed consent, and the trials were registered at ClinicalTrials.gov (identifiers:

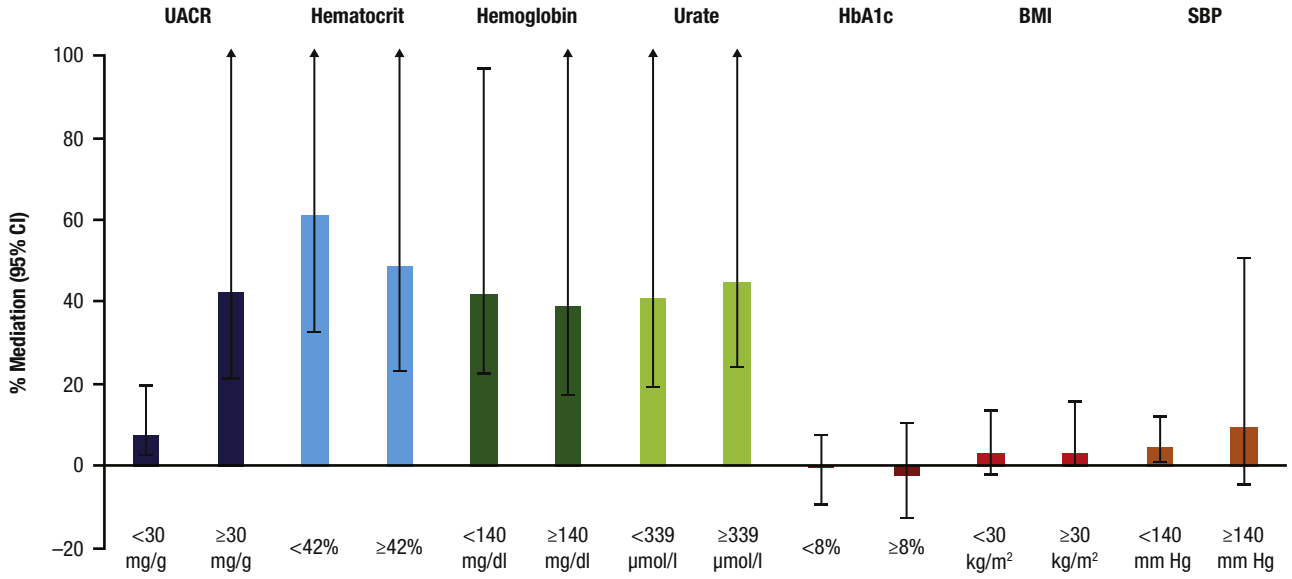

Figure 3 | Percentage of the mediating effect of potential mediators of the effect of canagliflozin on the composite kidney outcome across patient subgroups. BMI, body mass index; CI, confidence interval; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; UACR, urinary albumin:creatinine ratio.

NCT01032629 and NCT01989754). All procedures followed were in accordance with the Helsinki Declaration of 1964, as revised in 2013.

Participants

Participants were individuals with type 2 diabetes and an increased cardiovascular risk.[33] Participants were either 30 years of age or older with a history of symptomatic atherosclerotic cardiovascular disease, or 50 years of age or older with 2 or more of the following risk factors for cardiovascular disease: duration of diabetes of at least 10 years, SBP higher than 140 mm Hg while receiving 1 or more antihypertensive agents, current smoking, microalbuminuria or macroalbuminuria, or a high-density lipoprotein cholesterol level of less than 1 mmol/l (38.7 mg/dl). Participants were required to have an estimated glomerular filtration rate at entry of more than 30 ml/min per 1.73 m^2 of body surface area.

Randomization and study treatment

After a 2-week, single-blind, placebo run-in period, participants were randomized centrally through an interactive web response system using a computer-generated randomization schedule prepared by the study sponsor using randomly permuted blocks. Participants in CANVAS were assigned in a 1:1:1 ratio to canagliflozin 300 mg, canagliflozin 100 mg, or matching placebo, and participants in CANVAS-Renal were assigned randomly in a 1:1 ratio to canagliflozin or matching placebo, administered at an initial dose of 100 mg daily with optional up-titration to 300 mg from week 13. Participants and all study staff were masked to individual treatment allocations until the completion of the study. Use of other background therapy for glycemic and cardiovascular risk management was performed according to best practice instituted in line with local guidelines.

Follow-up evaluation

Participants were followed up after randomization by face-to-face follow-up evaluation with 3 visits scheduled in the first year and further visits scheduled at 6-month intervals thereafter, with alternating telephone follow-up evaluation and face-to-face assessments. The occurrence of hospitalization for kidney outcomes was evaluated at every scheduled follow-up evaluation.

Outcomes

The outcome studied in this analysis was the composite outcome of a sustained 40% reduction in the estimated glomerular filtration rate, end-stage kidney disease, or death resulting from kidney disease. A blinded end point committee independently adjudicated all potential kidney outcomes using rigorous definitions that were prespecified according to established criteria.[34,35]

Selection of potential mediators

A diverse set of biomarkers was measured at baseline and at multiple time points during the follow-up period. The mediators initially considered for investigation in this analysis were biomarkers that were believed to likely be changed by treatment with canagliflozin and associated with the risk of kidney outcomes. Potential mediators were grouped into those likely acting through effects on glycemia, vascular tone, lipids, kidney function, adiposity, volume status or hematopoiesis, acid–base balance, and serum urate (Table 1). Fasting plasma glucose level, urine pH, and ketonuria were measured in CANVAS, but not CANVAS-Renal, and were assessed in subsidiary analyses restricted to CANVAS participants. Ketonuria was assessed as a dichotomous variable (none vs. trace or more), but all other potential mediators were assessed as continuous measures.

Statistical analysis

The standard statistical method involves quantifying the effect of a potential mediating variable on the primary association between the exposure and the outcome of interest.[36,37] If the inclusion of the potential mediator in the primary model results in an attenuation of the strength of the association between a drug and an outcome, this is interpreted as the effect of the drug on the outcome being mediated by the biomarker of interest. Another widely used approach for assessment of mediation uses a product method.[38] For a biomarker to be eligible as a mediator, several conditions should be fulfilled. First, canagliflozin compared with placebo should exert an

effect on the biomarker of interest and, second, the post-randomization level of the biomarker should be associated with the risk of kidney outcomes.

The selection of potential mediators occurred on the basis of establishing the effect of canagliflozin versus placebo on the potential mediators using mixed models incorporating repeated measures of the potential mediator. The between-group difference was assessed using residual restricted maximum likelihood tests. The exception to this approach was for the evaluation of ketonuria in CANVAS, which was assessed using a logistic regression model because urinary ketones were recorded as a categoric variable. Associations of the potential mediator with the kidney outcome were determined from Cox regression models. In subsidiary analyses, the effect of canagliflozin relative to placebo was determined on the early change in the potential mediator by estimating the change in the potential mediator from baseline to first postrandomization measurements, which variously was made mainly between 6 and 18 weeks into the follow-up period.

Variables with skewed distributions were analyzed after log transformation (triglycerides, UACR, GGT). Fasting plasma glucose level, urine pH, and ketonuria were available only from CANVAS. Individuals without a baseline measure of the mediator of interest were excluded from the relevant analyses, as were individuals with no follow-up measurement and those with a baseline measurement who had kidney outcomes before a follow-up measurement was made.

The primary analyses were comparisons of hazard ratios from Cox survival models for the association between randomized treatment and the risk of kidney outcome, unadjusted and adjusted for each biomarker in turn. In each case, the percentage mediation was estimated as follows: $100\% \times \left(\frac{HR-HR_c}{HR-1}\right)$, where HR_c is the hazard ratio after adjustment for the biomarker and HR is the unadjusted hazard ratio.[39] The 95% CIs for the estimated percentage mediation were obtained using a 5000-iteration bootstrap resampling procedure. The combined potential mediating effect of multiple biomarkers was quantified using the same equation. Multiple mediator models were built by first selecting the biomarker with the largest percentage mediation value. Each remaining biomarker then was included, in turn, and the next biomarker that produced the greatest joint mediation was added to the existing model. This was repeated until 3 mediators were added, with the mediation effect reaching 100%, or 4 mediators were added in the multivariable model. Only 1 variable from each biomarker group was included in the multivariable analysis because the goal was to capture different mechanistic processes that were likely to mediate the effects of the drug.

To further test the robustness of the findings, we performed a secondary analysis using the product method under the counterfactual framework approach[40] for univariable assessments, and using nonlinear models (multiple additive regression trees and smoothing splines) for multivariable assessments that were able to account for collinearity between potential mediators. Briefly, the product method under the counterfactual framework divides the overall effect of a treatment on an outcome determined from a Cox regression model into direct and indirect components, which enables estimation of the proportion of the effect mediated by the biomarker of interest. For multivariable analysis based on linear models, correlation among mediators can lead to estimates of effect mediation that are greater than 100% and also may widen CIs. The use of nonlinear models is more robust to overestimations caused by collinearities.[41]

For evaluation of the mediating effects of early effects of canagliflozin, the statistical models were adjusted for the baseline value of the biomarker to control for regression to the mean. Statistical models exploring the long-term mediating effects by incorporating the average biomarker level were not adjusted for the baseline value because the baseline value already was included in the calculation of the average effect. To further explore the impact of baseline values of the potential mediator, mediation analyses were repeated in patient subgroups defined by median values or clinically relevant thresholds.

All analyses were performed using SAS version 9.4 (SAS; Cary, NC) and R studio version 1.1.463 (RStudio, Boston, MA). *P* values less than 0.05 were deemed significant.

DISCLOSURE

JL is a full-time employee of the George Institute for Global Health. BN is supported by an Australian National Health and Medical Research Council Principal Research Fellowship; holds a research grant for this study from Janssen; and has held research grants for other large-scale cardiovascular outcome trials from Roche, Servier, and Merck Schering-Plough; and his institution has received consultancy, honoraria, or travel support for contributions he has made to advisory boards and/or the continuing medical education programs of Abbott, Janssen, Novartis, Pfizer, Roche, and Servier. VP has received fees for advisory boards, steering committee roles, or scientific presentations from AbbVie, Astellas, AstraZeneca, Bayer, Baxter, Bristol-Myers Squibb, Boehringer Ingelheim, Dimerix, Durect, Eli Lilly, Gilead, GlaxoSmith-Kline, Janssen, Merck, Mitsubishi Tanabe, Mundipharma, Novartis, Novo Nordisk, Pfizer, PharmaLink, Relypsa, Retrophin, Sanofi, Servier, Vifor, and Tricida. DdZ has served on advisory boards and/or as a speaker for Bayer, Boehringer Ingelheim, Fresenius, Mundipharma, and Mitsubishi Tanabe; has served on steering committees and/or as a speaker for AbbVie and Janssen; and has served on data safety and monitoring committees for Bayer. BLN is supported by an Australian National Health and Medical Research Council Postgraduate Scholarship and a University Postgraduate Award from the University of New South Wales, and he has received travel support from Janssen. CA is an employee of the George Institute. RS is a full-time employee of Janssen Research & Development, LLC. RO is a full-time employee of Janssen Research & Development, LLC. KWM has received research support from Afferent, Amgen, Apple, Inc, AstraZeneca, Cardiva Medical, Inc, Daiichi, Ferring, Google (Verily), Johnson & Johnson, Luitpold, Medtronic, Merck, National Institutes of Health (NIH), Novartis, Sanofi, St. Jude, and Tenax; and has served as a consultant (speaker fees for continuing medical education events only) for Abbott, Ablynx, AstraZeneca, Baim Institute, Boehringer Ingelheim, Bristol-Myers Squibb, Elsevier, GlaxoSmithKline, Johnson & Johnson, MedErgy, Medscape, Mitsubishi Tanabe, Myokardia, NIH, Novartis, Novo Nordisk, Portola, Radiometer, Regeneron, Springer Publishing, and the University of California, San Francisco. HJLH is supported by a VIDI (917.15.306) grant from The Netherlands Organisation for Scientific Research; has served as a consultant for AbbVie, Astellas, AstraZeneca, Boehringer Ingelheim, Fresenius, Gilead, Janssen, Merck, Mundipharma, Mitsubishi Tanabe, and Retrophin; and has received grant support from AbbVie, AstraZeneca, Boehringer Ingelheim, and Janssen. The CANVAS Program was sponsored by Janssen Research & Development, LLC. Technical editorial assistance was provided by Kimberly Dittmar, PhD, and Elizabeth Meucci, PhD, of MedErgy, and was funded by Janssen Global Services, LLC.

DATA STATEMENT

Data from the CANVAS Program are available in the public domain via the Yale University Open Data Access Project (http://yoda.yale.edu).

ACKNOWLEDGMENTS

The authors thank all participants, investigators, and trial teams for their participation in the trial. The CANVAS Program was sponsored by Janssen Research & Development, LLC, and was conducted collaboratively by the sponsor, an academic-led steering committee, and an academic research organization, George Clinical. Analyses were performed by George Clinical and confirmed independently by the sponsor. All authors provided input through the development of this manuscript and approved the final version for submission. Technical editorial assistance was provided by Kimberly Dittmar, PhD, and Elizabeth Meucci, PhD, of MedErgy, and was funded by Janssen Global Services, LLC. Canagliflozin has been developed by Janssen Research & Development, LLC, in collaboration with the Mitsubishi Tanabe Pharma Corporation.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Number of measurements during follow-up evaluation of potential mediators.

Table S2. Effects of canagliflozin on biomarkers that might mediate the effect of canagliflozin on the composite kidney outcome for biomarkers that were measured in CANVAS but not CANVAS-R.

Table S3. Effects of multivariable adjustment for potential mediators of the effect of canagliflozin on kidney disease when fitted as changes measured immediately after taking the drug.

Table S4. Analysis of potential mediators of the effect of canagliflozin on kidney outcome using changes measured as average levels during follow-up evaluation using the product method under the counterfactual framework.

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